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(54) **Method of preparing a milk protein isolate.**

(57) A method of preparing a milk protein isolate by ultrafiltration of a milk derivative containing dissolved milk proteins, comprising ultrafiltration of a non-preheated or at most low pasteurized skim milk or mixture of skim milk and a whey containing solution at a pH ranging from 3 to 4, followed by diafiltration of the retentate, then increasing the pH to a value substantially within the neutral range, at which pH ultrafiltration is continued until a solids content in the retentate of at least 15% and in particular about 20%.

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This invention relates to a method of preparing a milk protein isolate from skim milk or from a mixture of skim milk and whey or whey protein concentrate.

Milk proteins are highly important. They are virtually indispensable in the composition of a large number of foods, such as sweets, bakery products and meats, in particular because of their palatability, high nutritional value and their favourable contribution to the texture of those foods. No other substances equalling milk proteins as regards properties are known for this purpose.

For different applications various milk protein products having different compositions are known, such as milk powders, whey powders and more or less purified to highly purified milk protein isolates.

Of the milk protein isolates the caseinates are most important and these proteins are particularly applied as emulsifier and stabilizer in foods like meats, soups and sauces.

The best known method of preparation for those caseinates comprises an acid precipitation of the casein proteins at a pH of 4.6, followed by washing the precipitation and redissolving the protein coagulum by increasing the pH. By using suitable bases there can be prepared sodium, potassium, ammonium and calcium caseinates.

In this method, i.e. carried out without heating, the whey proteins remain dissolved, so they are not recovered too. Since these proteins are not coprecipitated, the protein loss will be about 20%. Accordingly, a number of processes have been developed for recovering whey protein together with the caseinates as so-called total milk protein powder.

Dutch patent application 8204923 discloses a method of preparing so-called total milk protein powder, which method is not essentially different from the known method for the preparation of a so-called coprecipitate, i.e. casein together with the whey proteins. The product thus obtained proves to be poorly soluble, so that this product is not always applicable in practice.

The literature describes improvements in this known method (cf PCT/US81/01357). According to these proposals skim milk is heated and cooled at an increased pH, followed by adjusting the pH to a value of 4.6 and recovering the precipitate consisting of a coprecipitate of casein and whey protein. The product thus obtained has a solubility equal to that of sodium caseinate. Yet this method also has some disadvantages. First of all an increased yield of milk protein of 20% is not obtained. After separation of the coagulum the liberated serum still contains 0.35% protein nitrogen, which, after correction for the NPN (non-protein nitrogen), corresponds to a loss of at least 5%. Moreover, the solution of the product obtained has a relatively high viscosity, which is to be ascribed to the high degree of denaturation of the whey proteins.

In the method of the invention skim milk or a mixture of skim milk and whey or whey concentrate is ultrafiltered at a pH ranging from 3 to 4, followed by subjecting the retentate to a diafiltration and then increasing the pH to a value of about 6.5, at which pH the ultrafiltration is continued until a solids content in the retentate of at least 15% and in particular about 20%.

Surprisingly, it has been found that by ultrafiltering skim milk below the isoelectric point of the milk proteins both the casein and the whey proteins can be isolated from the milk or from the mixture of milk and whey or whey concentrate at a relatively high capacity. An important advantage obtained using the method of the invention is that at a pH ranging from 3 to 4 the casein proteins are suboptimally soluble, so that the flux through the membrane can remain relatively high without the occurrence of clogging. Thus, with that high flux a concentration factor of 2.5 is obtainable. An additional advantage obtained by using the method of the invention is a very low calcium retention, so that a very low calcium content is obtained in the product.

It is observed that ultrafiltration at a low pH for the preparation of milk protein products is known per se and is described in British patent 1,362,502. The object of the method described is to improve the reconstitution properties and to prevent off-flavour owing to heating and drying a protein coagulum obtained in a classical manner. According to the method described a milk product containing precipitated casein is acidified to a pH below the isoelectric point by adding a certain amount of an acidified milk derivative. By means of ultrafiltration the protein content can be increased. The casein is then dissolved, followed by drying in a classical manner. Diafiltration is not applied. The method described here has the disadvantage that for redissolving the casein heating of the mixture is necessary, unless the pH has a very low value. This heating results in possible off-flavours, certainly if the mixture also contains whey proteins.

In the method of the invention the degree of isolation can be adjusted by applying the diafiltration after the first ultrafiltration, which is carried out at the same temperature and pH as in the first ultrafiltration. From a nutritional viewpoint KOH is preferably used to increase the pH after the first ultrafiltration. The second ultrafiltration is preferably continued until a solids content of 20%.

As observed above, the selection of the pH adjustment is highly important in the method of the invention, in particular as regards the calcium retention, the viscosity and solubility of the retentate as well as the bacteriological quality of the products obtained.

With respect to the viscosity it is observed that this is higher as the pH is lowered and as a result of this increased viscosity the capacity of the ultrafiltration process will decrease. The solubility also increases according as the pH is more remote from the isoelectric point, as a result of which the capacity also decreases, which, after all, is inversely related to the solubility of the protein. An optimum pH in the range, i.e. in which the combination of the parameters partly opposing each other is optimal, is about 3.

Graphs 1, 2 and 3 show the relation between the flux over the membrane and the pressure drop per stage during the ultrafiltration of acidified skim milk, at different pH's. The experiments were conducted with a two-stage Abcor system. The different flux values relate to a continuous process in which skim milk was ultrafiltered at a pH of 3, 3.5, and 4. The flux of both the first and the second stage is shown in the graphs at a concentration factor and a volume reduction of respectively 2.5 and 60%. The data show that during a life test of 6 hours at pH 3 the average flux is at least 10% higher than at both pH 3.5 and pH 4.0. Graph 4 compares the effect of the pH on the capacity in a batchwise process. In the relevant experiments the membrane permeability (flux) of skim milk was monitored at 50°C during concentration until a factor of 2.5. These results also show that during concentration a pH of about 3 is the optimum value.

Another important parameter in the method of the invention is the temperature at which the ultrafiltration is applied. The selection of this temperature is determined, on the one hand, by the material from which the membrane is made and, on the other hand, by its effect on the product properties. To most of the membranes a maximally allowable temperature ranging from 50 to 60°C applies. As regards the product properties, it applies that until a temperature of 70°C the viscosity decreases at increasing temperature, but that at a temperature of about 70°C or more the whey proteins are denatured, so that the viscosity increases.

It has further been found that in connection with the capacity of the method of the invention the pH reduction to a value below the isoelectric point of casein proteins is preferably effected at low temperatures, preferably at a temperature ranging from 0 to 5°C. As is well known, the casein proteins do not coagulate at temperatures below 5°C (cf Brulé & Lenoir in Cheese Making Sci. and Techn. (1987) Ed. A. Eck, page 15). If, however, the pH is lowered at a temperature of 50°C, the casein proteins momentarily flocculate when passing the isoelectric point (pH about 4.5) and this flocculation does not prove to be immediately reversible at the pH of ultrafiltration (about 3). Accordingly, the result of such a pretreatment is a long dissolving time of the flocculate and/or a low capacity (flux).

By using the method of the invention there is obtained a milk protein isolate in which the casein proteins are contained in the form of a caseinate and the serum proteins in native form. The product thus obtained has a neutral flavour and a relatively low viscosity. The method further leads to a product in which both the lactose content and the calcium content is strongly reduced, in particular to respectively below 2% and 0.4%, based on the solid matter. This shows that there is thus obtained a protein isolate of excellent quality.

The method of the invention can in principle be carried out as a continuous process. By properly adjusting the capacity of the ultrafiltration process both at low and at neutral pH, skim milk can be processed in one process step to a TMP concentrate containing more than 90% protein, based on the solid matter.

According to a preferred embodiment of the method of the invention the retentate is subjected, between the ultrafiltration at low pH and that at higher pH, to a limited proteolytic hydrolysis. Preferably, use is made of a protease active at low pH, which is deactivated by increasing the pH to neutral value. The incubation may be effected in the form of a holding heater placed between the low pH stage and the neutral pH stage. It has been found that an enzyme contact time of about 15 minutes with a proteolytic enzyme of Aspergillus saitoi, commercially sold under the name of Molsin, gives a milk protein hydrolysate having excellent foaming characteristics. The invention will be further illustrated by the following Examples.

Example I

1000 kg skim milk were acidified to a pH value of 3 with stirring at a temperature of 5°C, by means of a 5N hydrochloric acid solution. By using a three-stage ultrafiltration installation with Abcor high-flow membranes having sizes of respectively 7 m2, 10 m2, and 7.5 m2 the milk was concentrated. Subsequently, the milk was concentrated in a continuous process at pH 3 and a temperature of 50°C until a concentration factor of 2.5 and then diafiltered at the same pH and temperature in a second and third stage, using a double amount of water (200%).

Thus, 480 kg retentate having a solids content of 9% and a protein content of 6.7% were obtained. This retentate was diluted twice with water and the pH was adjusted to a value of 6.5 by means of a liquor injection (5N caustic soda solution).

The dilute retentate was then concentrated until a concentration factor of 7.5 at a temperature of 50 °C, using a three-stage installation.

The retentate thus obtained was directly spray dried. The powder obtained had a protein content of more than 90%, based on the solid matter, and, in addition to the casein proteins, also contained the whey proteins in undenatured form.

The method described can be schematically shown as follows:

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1000 kg skim milk (1) pH 3
            |
   400 kg retentate (2)
            |
diluted twice with water pH 6.5
            |
   480 kg retentate (3)
            |
   152 kg retentate (4)
            |
     30 kg powder (5)
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In the following table the composition of the products obtained during the different phases of the method is schematically shown:

Product( )	pH	% ds	% protein	% lactose	% salts	% calcium	protein/ ds
milk (1)	3.1	9.35	3.4	4.8	0.76	0.12	34.5
ret (2)	3.1	14.8	8.4	5.0	0.76	0.12	56.9
ret (3)	6.6	9.0	6.7	1.75	0.3	0.04	74.5
ret (4)	6.5	18.6	17.1	0.7	0.8	0.07	91.9
powder (5)	6.5	96.7	88.9	3.4	4.2	0.37	91.6

Example II

This Example describes the preparation of a protein product having a high foamability.

By using a laboratory ultrafiltration equipment of the mark AMICON, type DH2, a spirally wound membrane S1 (cut-off = 10,000 Dalton) 2500 ml pretreated skim milk were concentrated.

The pH of the thermized milk was adjusted at a temperature of 5 °C to a value of 3, using 4N hydrochloric acid. Subsequently, the temperature was increased batchwise to 50 °C, followed by concentrating the milk with the above ultrafiltration device until a concentration factor of 2.5. Then the retentate was subjected to a diafiltration step, using a double amount of water (200%), at equal pH and temperature.

Subsequently, a protease active at this pH was added to this retentate (the enzyme preparation Molsin, orginating from the fungus Aspergillus saitoi) and after a certain (specified hereinafter) incubation time the protein splitting enzyme was deactivated by increasing the pH to a value of 6.5, by means of a 4N sodium hydroxide solution.

Subsequently, the retentate/substrate was diluted twice with deionized water and further ultrafiltered until a concentration factor of 3.

The method was repeated with the protease at different incubation times, i.e. of 15, 30, 60 and 130

minutes.

The retentates finally obtained were freeze dried, and they were compared as regards their foaming characteristics.

The degree of hydrolysis was determined by a TNBS method, which enabled determination of the increase in the number of broken peptide bonds (percentage DH).

In the following table the incubation time, percentage DH, and the foam expansion are given.

Incubation time (min.)	DH (%)	Foam expansion (%)
0 (blank)	0	1220
15	0.7	2160
30	1.7	2760
60	2.5	2760
130	3.3	1840

Claims

1. A method of preparing a milk protein isolate by ultrafiltration of a milk derivative containing dissolved milk proteins, characterized by ultrafiltration of a non-preheated or at most low pasteurized skim milk or mixture of skim milk and a whey containing solution at a pH ranging from 3 to 4, followed by diafiltration of the retentate, then increasing the pH to a value substantially within the neutral range, at which pH ultrafiltration is continued until a solids content in the retentate of at least 15% and in particular about 20%.

2. A method as claimed in claim 1, characterized by carrying out the first ultrafiltration at pH 3.

3. A method as claimed in claims 1-2, characterized by carrying out the second ultrafiltration at pH 6.5.

4. A method as claimed in claims 1-3, characterized by carrying out the ultrafiltration at a temperature of not more than 50 °C.

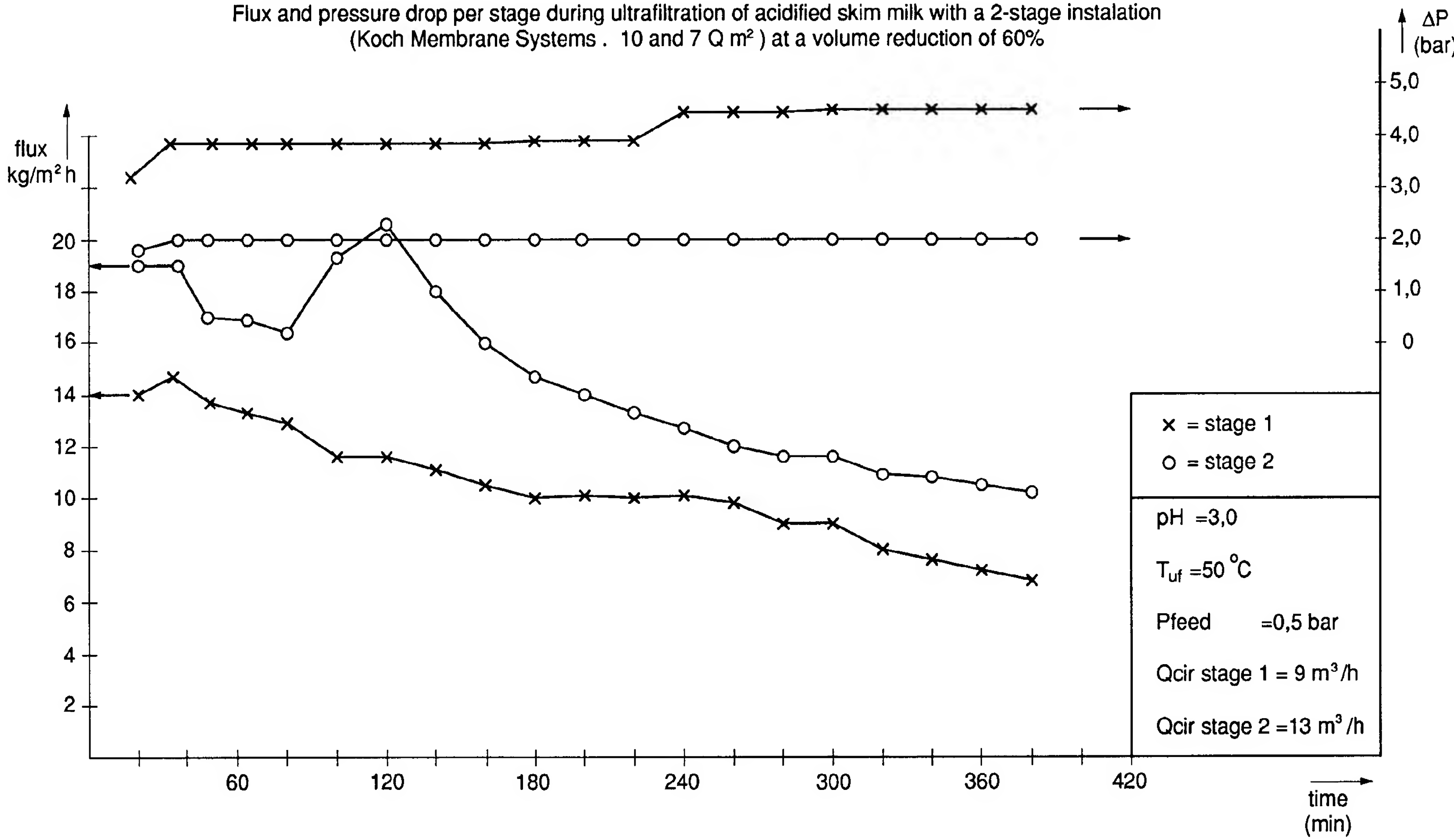
5. A method as claimed in claim 1, characterized in that, prior to the first ultrafiltration, the pH decrease of the skim milk or of the mixture of skim milk and whey containing solution takes place at a temperature of not more than 5 °C.

6. A method as claimed in claims 1-5, characterized in that between the ultrafiltration at low pH and the ultrafiltration at higher pH the retentate is subjected to an enzymatic hydrolysis.

7. A method as claimed in claim 6, characterized by carrying out the enzymatic hydrolysis with a proteolytic enzyme for about 15 minutes.

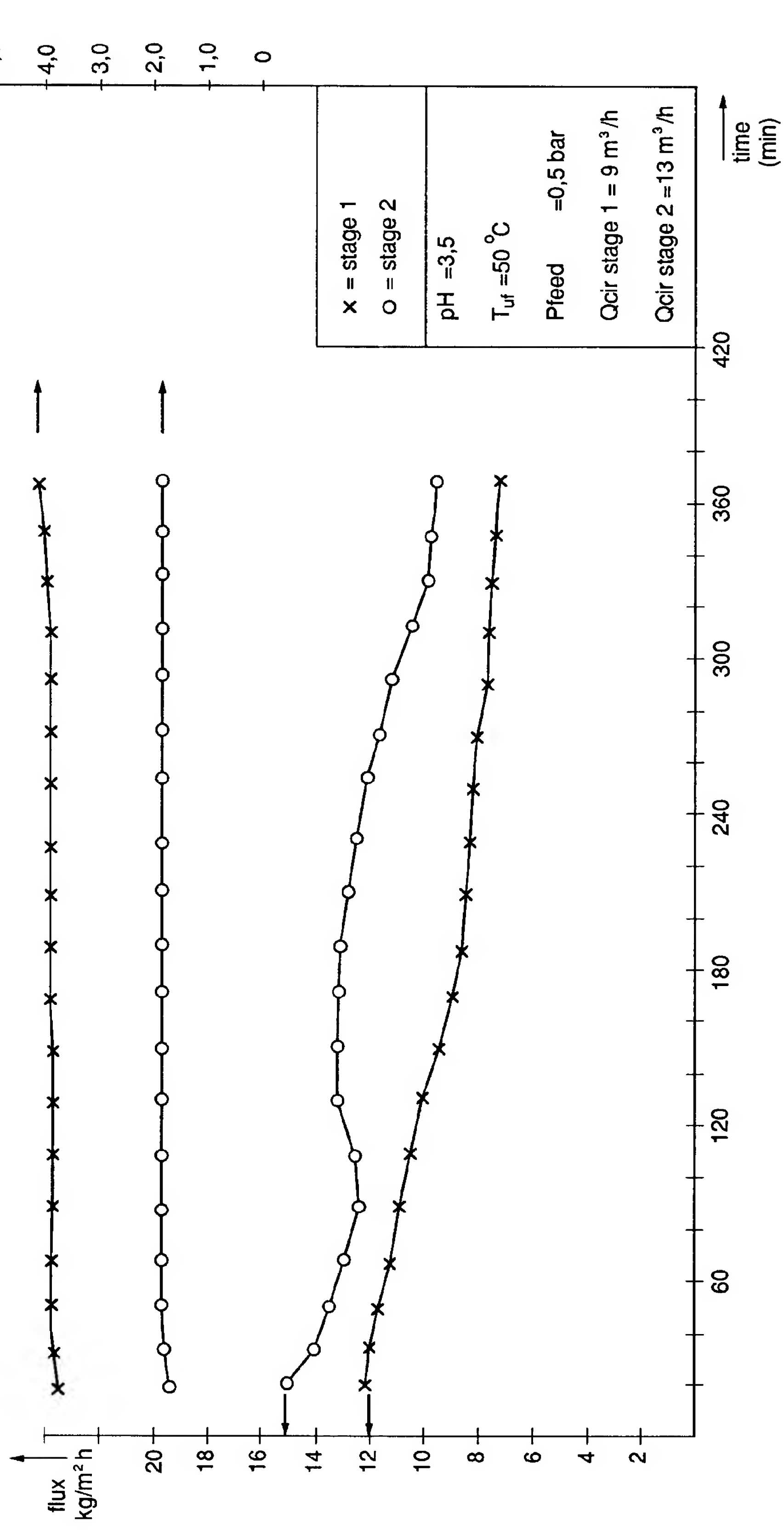
Graph 1

Flux and pressure drop per stage during ultrafiltration of acidified skim milk with a 2-stage instalation (Koch Membrane Systems . 10 and 7 Q m²) at a volume reduction of 60%



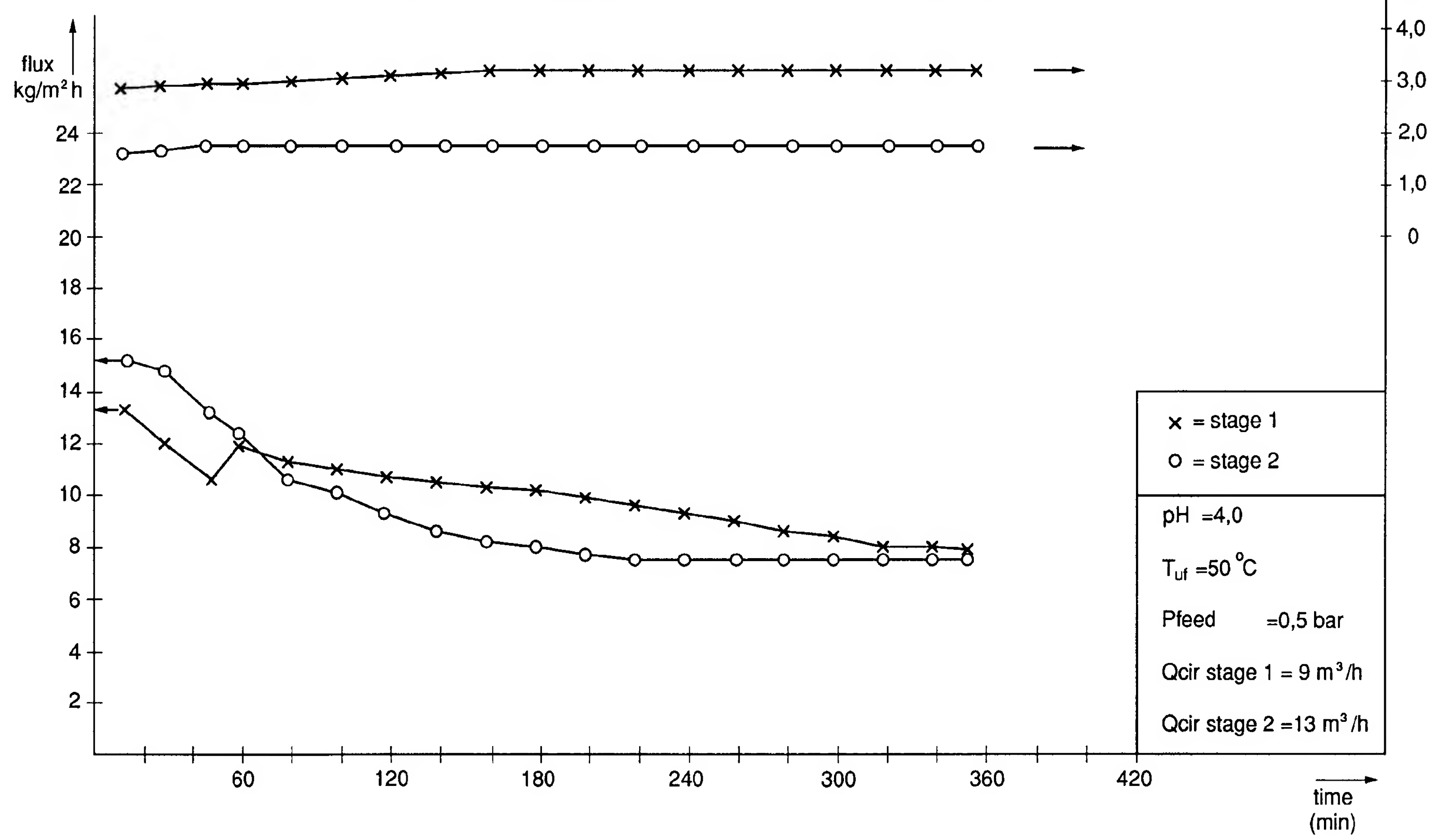
Graph 2

Flux and pressure drop per stage during ultrafiltration of acidified skim milk with a 2-stage instalation (Koch Membrane Systems . 10 and 7 Q m²) at a volume reduction of 60%



Graph 3

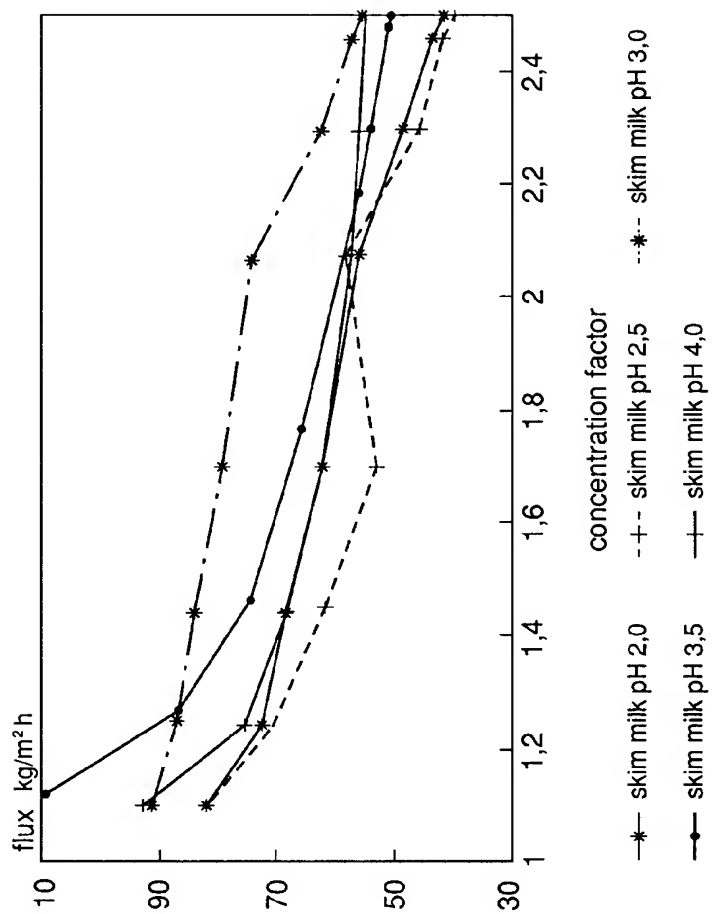
Flux and pressure drop per stage during ultrafiltration of acidified skim milk with a 2-stage instalation (Koch Membrane Systems . 10 and 7 Q m²) at a volume reduction of 60%



Graph 4

Flux measurement as a function of the concentration

Skim milk i.p. membrane spiral w. 10000 cut-off



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EUROPEAN SEARCH REPORT

Application Number

EP 91 20 1898

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	NL-A-7 603 369 (STICHTING BEDRIJVEN VAN HET NEDERLANDS INSTITUUT VOOR ZUIVELONDERZOEK) * Claims 1-8; example IV; page 4, paragraph 2 *	1,2,4	A 23 C 9/142 A 23 J 1/20 A 23 J 3/34
A	GB-A-1 362 502 (J.A. MEGGLE) * Claims 1,3,4,7,8; example 1 *	1,2,4	
A	EP-A-0 165 105 (LAITERIES E. BRIDEL) * Claims 1,2,3,4,5; page 2, lines 16-27 *	1,2,4,5	
A	NETHERLANDS MILK & DAIRY JOURNAL, vol. 32, no. 2, 1978, pages 80-93; J. HIDDINK et al.: "Removal of milk salts during ultrafiltratin of whey and buttermilk" * Pages 80,82-83,86-87 *	1,2,4	
A	JOURNAL OF DAIRY SCIENCE, vol. 63, no. 2, 1980, pages 228-234; C.A. ERNSTROM et al.: "Cheese base for processing. A high yield product from whole milk by ultrafiltration" * Page 228, column 1; pages 229,231-232 *	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
A	FR-A-2 367 431 (CENTRALE LAITIERE DE HAUTE NORMANDIE ET J. MONTIGNY) * Claim 15; page 5, line 28 - page 6, line 16 *	1,3,4	A 23 C A 23 J
A	EP-A-0 033 686 (INRA) * Claims 1,3,4; figures 1,2 *	6,7	
A	WO-A-8 703 786 (E. SAMUELSSON) * Claims 1,3; pages 6-9 *	6,7	

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	28 October 91	DESMEDT G.R.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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